ARIZONA GAME AND FISH DEPARTMENT
HABITAT PARTNERSHIP PROGRAM
HABITAT ENHANCEMENT AND WILDLIFE MANAGEMENT PROPOSAL

PROJECT INFORMATION

Project Title: Establishment of a Forensics Genetic Database for Big Game	**Project No. 10-705**
Region/GMU: Statewide	**HPC: N/A**

Project Type: Wildlife Law Enforcement

Project Description:

This project will initiate the establishment of a genetic database that is scientifically sound and statistically significant and will provide genetic data for management and regional assignment of big game species in Arizona. The data will be used in making informed management recommendations and for the future establishment of a wildlife forensics laboratory once significant funds are established. DNA/RNA material will be initially collected from mountain lion samples (whole blood and/or tissue) that are provided by AZGFD and cooperators of the U of A Conservation Genetics Lab located in Tucson at the U of A Cooperative Fish and Wildlife Research Unit, USGS School of Natural Resources and the Environment. Samples will be collected, stored, processed and analyzed from locales throughout the geographical range of the mountain lion. This initial project is the perquisite pilot to a larger vision of a statewide forensics lab for **all big game species**. See the attached companion PDF file entitled "Arizona Wildlife Forensics Unit & Laboratory" for additional information.

Possible Funding Partners: State and federal natural resource management agencies

Implementation Schedule: **Beginning: March 2011** **Completed: December 2012**	**NEPA Compliance: (if applicable)** **Not Applicable_X__** **Completed:** **Yes ____ No ____** **Projected:** **Completion Date:**

PROJECT FUNDING

SBG Funds Requested: $7,500 each year for two years for a total of $15,000. Support for this amount is requested to be funded by an appropriate portion from all the individual species' funds (or possibly more appropriate from the Super Raffle).

Cost Share Funds: $2,500 each year for two years from the Wildlife Theft Prevention Fund, AZGFD Law Enforcement Branch (contact: Gene Elms, Law Branch Chief)

Plus: Additional Funding Sources: NSF-IGERT fellowship program in Genomics – Graduate student stipend $30,000 per year and $1,000 towards materials for research in genomics; AZ-CFWRU USGS – and in-kind salary for Principal Investigator $4,625 (Melanie Culver).

Total Project Costs: $90,250 over two years

PARTICIPANT INFORMATION

Applicant: Melanie Culver, Assistant Professor, Wildlife Conservation and Management Program Assistant Leader, AZ Cooperative Fish and Wildlife Research Unit **Telephone:** culver@ag.arizona.edu	**Address:** **USGS School of Natural Resources and the Environment University of Arizona** **213 Biosciences East** **Tucson, AZ 85721**

Office 520-626-3775 **Lab 520-626-1636** **Fax 520-621-8801** http://www.cals.arizona.edu/research/azfwru/melanie/	
AGFD Contact and Phone No. - Ron Thompson, Game Branch 623 236-7354 **(If applicant is not AGFD personnel)**	
Coordinated with: Ron Thompson and Gene Elms, Arizona Game and Fish Department	**Date: 8/31/2010**
Applicant's signature: Melanie Culver	**Date: 8/30/2010**

SEND COMPLETED APPLICATIONS TO:
AZ Game and Fish Department
Attn: Game Branch
5000 W. Carefree Highway
Phoenix, AZ 85086
rgregory@azgfd.gov

WAS PROJECT PRESENTED TO THE LOCAL HPC? YES _____ NO ___X___

HAS PROJECT BEEN SUBMITTED IN PREVIOUS YEARS? No IF SO WAS IT FUNDED?

NEED STATEMENT/PROBLEM ANALYSIS:

The persistent demand for wildlife specimens, parts, and products continues to stimulate illegal take of wildlife via unlawful activities by poachers and criminals that stand to make financial gains from unlawful commercialization of wildlife or their parts. Investigations of wildlife crime cases are essential to wildlife protection and management, and often require detailed information from intrinsic evidence such as blood, hair and tissue. To enhance investigative processes, a specialized unit that consist of a dedicated service laboratory, particularly in generating and providing robust science supported evidence to strengthen presentations in court trials. Prosecution or defense of a wildlife criminal is often based on critical pieces of evidence brought about by analysis of the chain of evidence in custody. A unit specializing in wildlife forensics will provide integral support services in this analysis. Moreover, the increasing demand for investigative services, such as sample testing and identification have restricted Arizona's use of the USFWS National Wildlife Forensics Lab at Ashland, OR to cases only involving threatened and endangered wildlife species

PROJECT OBJECTIVES:

The objectives of this project are to:

1) Establish a database of DNA material and genomic profiles that are capable of identifying specific regions or locales from which mountain lions have originated from. This is accomplished only by using a large scale molecular analysis of a species' genome that is capable of matching potential associations with previously identified environmental selection by making spatial (Arizona populations vs. other populations) and temporal (current, ~20 year-old tissue and blood samples, and museum samples) comparisons. The genes in these genomic regions will be identified by referencing the current annotated genome sequence from the domestic cat (*Felis catus*).
2) Establish a model to be used in an accredited forensics lab by which DNA material from all big game species will eventually be housed and forensically analyzed in solving and prosecuting criminal wildlife cases.

PROJECT STRATEGIES:

Expected Benefits/Outcomes: These candidate genes will serve as some of the first potential "markers" for evidence of similarity by region in Felidae (cat family), and may then potentially be extended to other species as well. A final report

summarizing the finding of this study as well as at least one peer reviewed scientific journal publication will result from this study.

Methods: We will use SNPs (single nucleotide polymorphisms) to genotype individual pumas from different spatial (geographic) and temporal populations. SNPs have been suggested to be the marker of choice for future research in conservation genetics (1). The SNPs will be identified by next-generation sequencing of messenger RNA derived from puma blood and of amplified DNA fragments. Depending upon the number of SNPs identified, a custom assay will be developed to genotype 30-1500 SNPs. This assay will be high-throughput, meaning nearly 400 puma DNA samples can be genotyped simultaneously (in a single reaction). The order of magnitude increase in the number of markers and cost per genotype decrease (~$.12/SNP compared to >$1/microsatellite) allows for a high-resolution examination of puma population structure, selection, and other population genetic factors. Identification of candidate genes has been done using SNP genotyping with as few as 20 samples (2). The candidate genes associated with populations of puma predicted to be experiencing larger effects from climate change will be identified and eventually sequenced. Further studies using these candidate genes will be done to support the hypothesis that these genes are indicative of selection relative to climate change (or other environmental factors).

Partners: Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD; U.S. Geological Survey, Reston, VA; Arizona Game and Fish Department, Phoenix, AZ; Arizona Research Laboratories, Univ. of Arizona, Tucson, AZ

Budget Needs: $20,000 of funding is required to cover materials and supplies to perform laboratory experiments for genome analysis of puma samples.

PROJECT LOCATION: Culver Conservation Genetics Laboratory – lab analysis (no field component)

LAND OWNERSHIP AT PROJECT SITE (Please state specifically if PRIVATE PROPERTY and provide landowner's name): N/A: samples have been collected and archived by AZGFD and collaborators.

IF PRIVATE PROPERTY, IS THERE A STEWARDSHIP AGREEMENT BETWEEN THE LANDOWNER AND THE DEPARTMENT? N/A

HABITAT DESCRIPTION: Mountain lion habitat throughout Arizona and throughout the range of the mountain lion.

ITEMIZED USE OF FUNDS:

Item	**Total cost over 2 yrs**
Salary (including benefits and student expenses):	$60,000 student stipend
Salary (including benefits)	$9,250 for advisor
Lab expenses	
Project funds from IGERT	$2,000
Materials and supplies	
DNA extraction	$2,000
PCR amplification	$5,000
SNP analysis	$10,000
Plastic ware	$1,500
Buffers and Reagents	$1,500
Total	**$91,250**

LIST COOPERATORS AND DESCRIBE POTENTIAL PARTICIPATION:

Coordination to collect and deliver archived mountain lion samples to U of A: Ashwin Naidu (U of A), Bob Fitak(U of A), AZGFD - Brian Wakeling, Ron Thompson, Rick Langley; Jon Hanna; Tom McCall; Erin Butler; Bob Henry; Jim Heffelfinger

PROJECT MONITORING PLAN:
N/A

PROJECT MAINTENANCE:
U of A will provide a one-year and final report to AZGFD and partner funding sources to facilitate dissemination of information and investigative implementation. Annual reports will provide both detailed explanations and a plain language summary and interpretation of the findings will be provided so that wildlife biologists and managers can understand the content and implications of the genetic findings.

The database and genetic markers developed will be maintained into the future unless release by AZGFD.

PROJECT COMPLETION REPORT TO BE FILED BY: The completed project report is due two years following receipt of contract for funding and receipt of necessary samples.

WATER DEVELOPMENT PROJECTS (see attached worksheet):
N/A

TREE SHEARING (AGRA-AXE, PUSH) PROJECTS (see attached worksheet):
N/A